EXHIBIT 2

4.3 Analysis of western blot images in 3rd row of Figure 2 from NIH grant proposal 5R44AG057329-03

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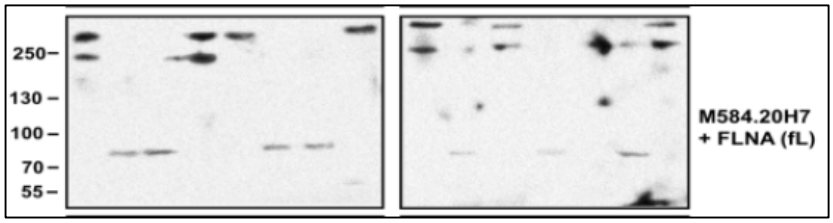
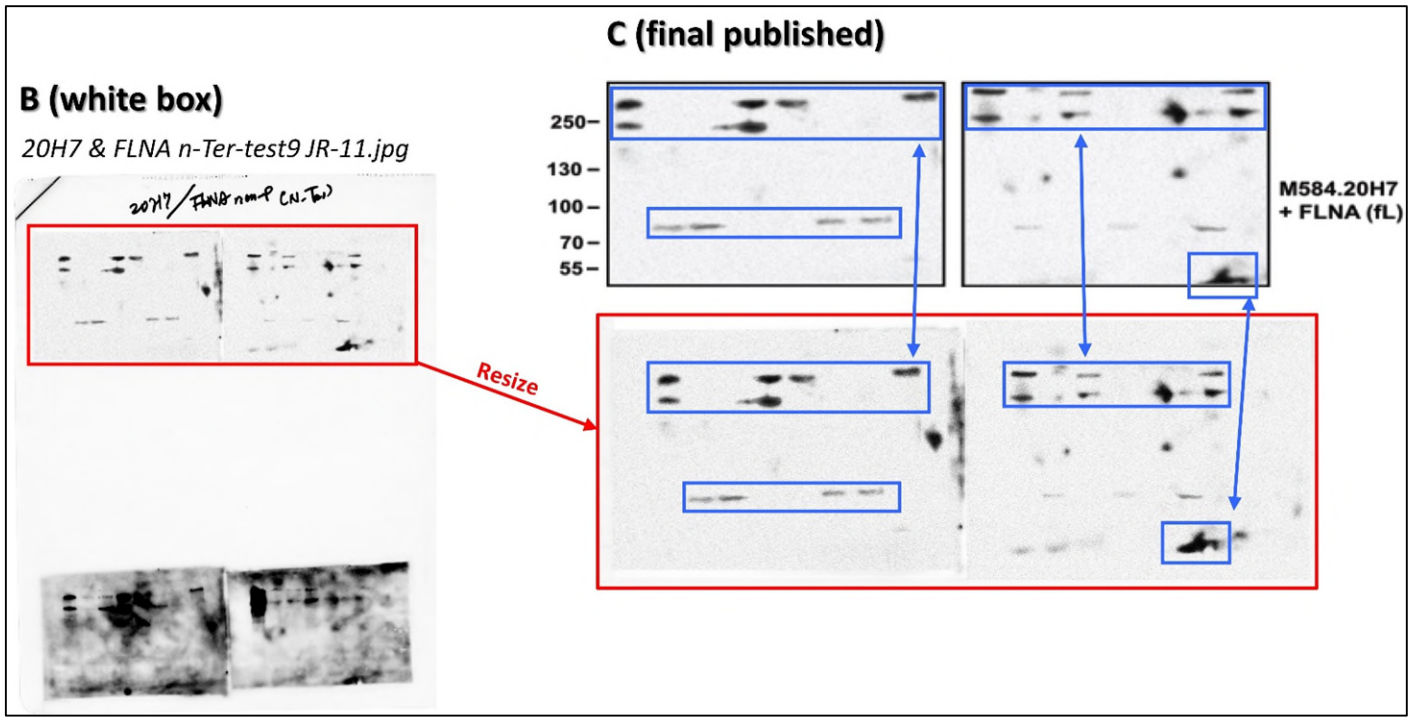
The 3rd row blot from Figure 2 of the proposal is shown here. The layout of blots is similar to the other row: Each lane represents a patient plasma sample, 8 samples in the left panel, 7 in the right. In this case the blots are probed with a mixture of both a custom in-house antibody (M584.20H7) plus an unnamed commercial antibody that reportedly binds to full-length filamin A (FLNA).



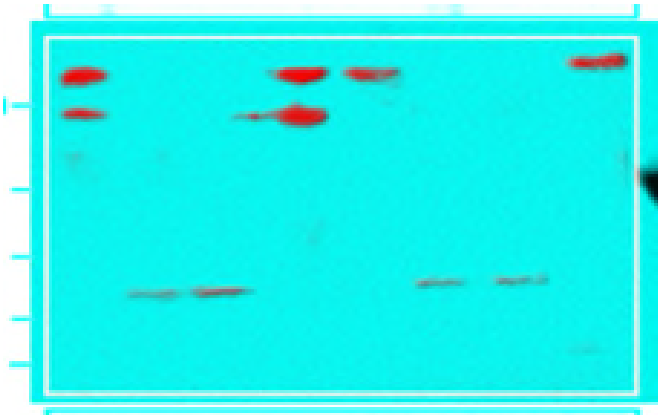
<u>Stage 1.</u> Reverse analysis

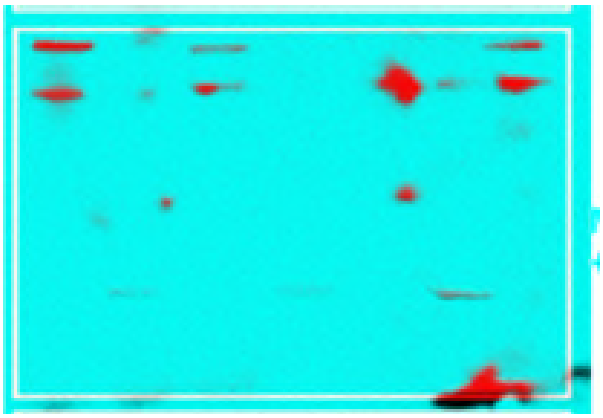
This stage asks the following questions: **Q1.** Can the final published image (C) be traced to a white-box image (B) and/or a raw image (A)? **Q2.** Can the final pattern of bands seen in the published image (C) be traced to the source images?

The file *"20H7 & FLNA n-Ter-test9 JR-11.jpg"* appears to contain an image that is the source for the 3rd row blots in Figure 2. As shown below, numerous features upper portion of this file (outlined in red) map to features in the 3rd row of Figure 2 (highlighted in blue). As such, the bands in the final figure (C) can be traced to the white box image (B).

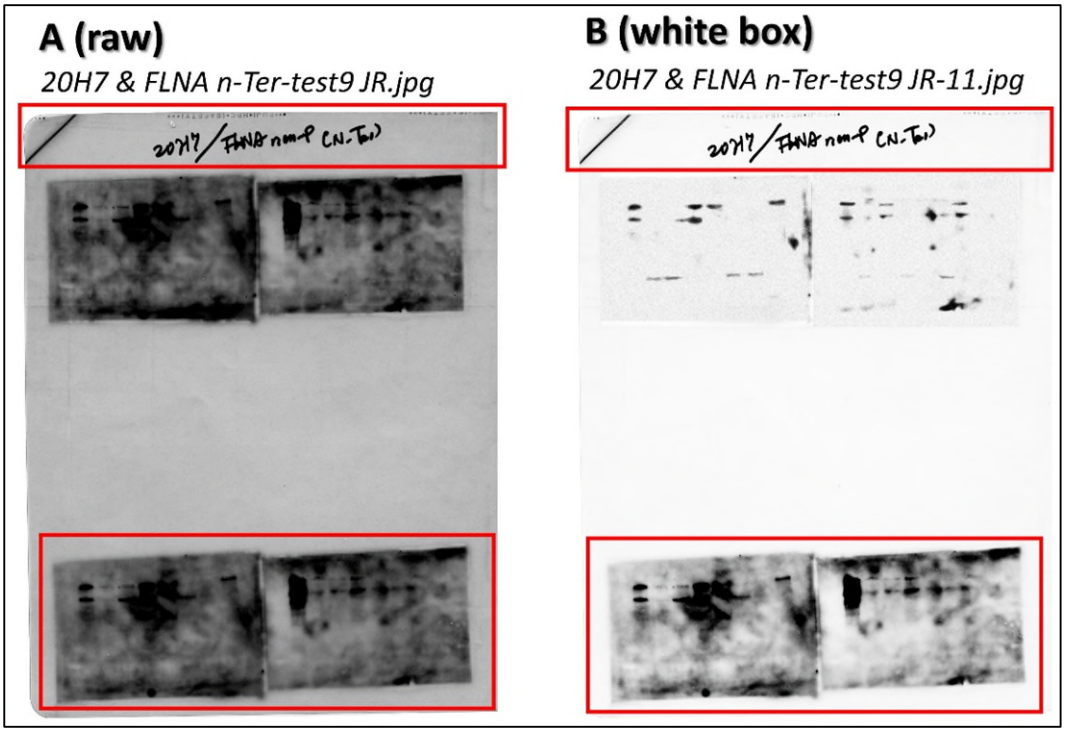


This lineage is confirmed below, with an overlay of the relevant parts of the two images using the ORI *forensic droplets* in Adobe Photoshop (https://ori.hhs.gov/droplets). Each image is pseudo-colored using a gradient map, with the result that overlapping features appear in red.





In addition to *"20H7 & FLNA n-Ter-test9 JR-11.jpg"*, an accompanying file is *"20H7 & FLNA n-Ter-test9 JR.jpg"*, which appears to contain an image of the same piece of film without the white box feature. Note that in this specific example, the image being referred to as "white-box" does not actually have a white box imposed on the raw image. Instead, it appears the whole image has been significantly brightened relative to the raw image. Nevertheless, numerous shared features (highlighted in red) including handwriting at the top, and the blots in the lower portions of the images, allow us to infer that both images were obtained from the same physical piece of film. It is concluded that the raw image (A) and the white-box image (B) share a common origin.



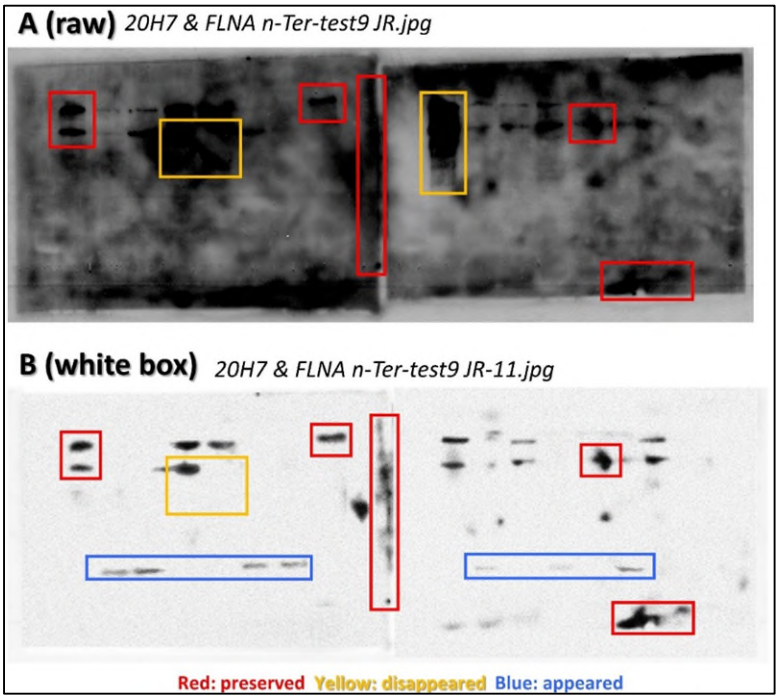
Next, we ask where the white box features in (B) originated? Can any of the bands or other features in the white box image (B) be traced to underlying features in the raw image (A)?

As seen on the next page, some bands and features are preserved between A and B (highlighted red), while others disappear (highlighted yellow), and others appear from nowhere (highlighted blue).

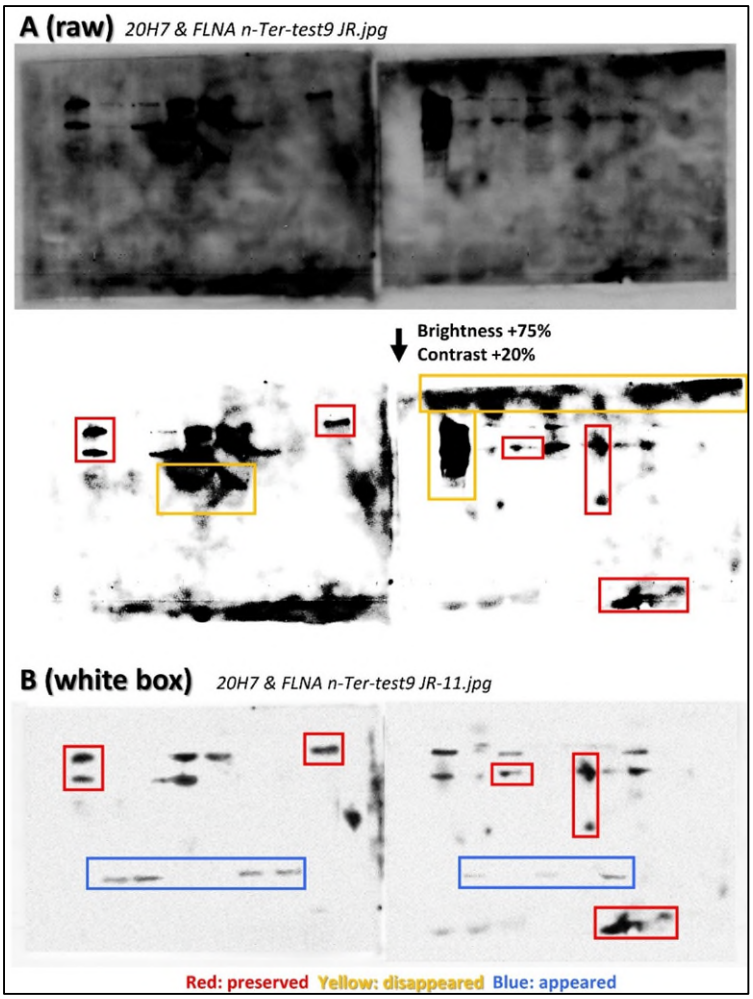
Importantly, all of the bands in the lower part of the image (at the molecular weight allegedly corresponding to the fragment of FLNA, 90 kDa) are nowhere to be seen in the raw image (A). Thus, it is concluded that the pattern of bands seen in the white box image (B), and therefore also in the final published image (C), cannot be traced to any matching features in the raw image (A).

Stage 2. Forward analysis

This stage asks (**Q3**) whether the source images can be manipulated using accepted techniques to recreate the final image? To accomplish this, the raw image (A) *20H7 & FLNA n-Ter-test9 JR.jpg* was subjected to brightness/contrast adjustment, to determine whether features in the white-box image (B) *20H7 & FLNA n-Ter-test9 JR-11.jpg*, could be recreated. This manipulation adhered to the fundamental rule of image processing – any adjustment (contrast, brightness, saturation, color, transparency, etc.) must be applied evenly to the entire field of view. It is unacceptable to adjust selected portions of a blot image such as individual bands.



As shown below, taking the raw image (A) and increasing brightness by 75% and contrast by 20% yields an image with clear bands on a pale background that looks similar to the white-box image (B). There are common features between the adjusted raw image and the white-box image (highlighted red). But, other features have disappeared (yellow), and yet more features have appeared from nowhere (blue).

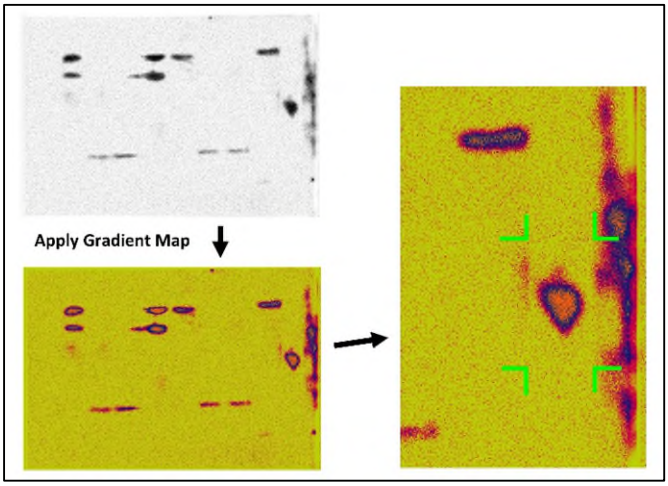


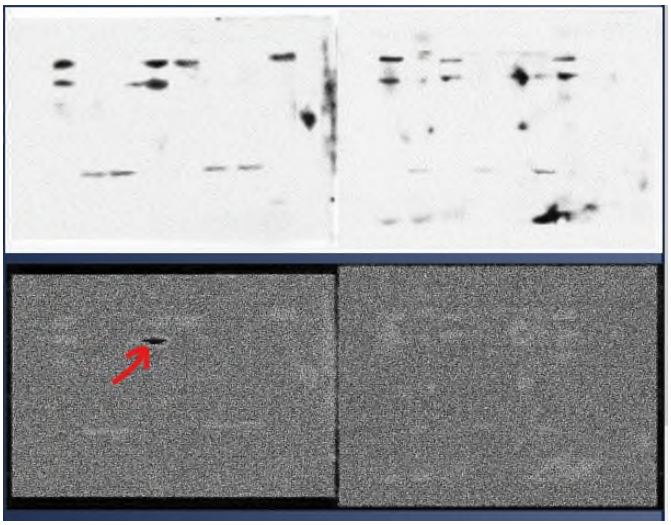
Importantly, many of the "smudges" or other irregularities along the edges of the blot images have been preserved (e.g. the "shark-like" profile in the lower right corner). This further connects the images, indicating a common source.

Several potential explanations for the appearance of bands highlighted in blue can be refuted. The box cannot have arisen from digital enhancement of an area in the raw image, because there is essentially no information available to enhance in the corresponding area of the blot. The white box bands cannot have originated from a different exposure of the blot film, since this would result in a separate piece of film, and as already established the raw image and the white box image are the same piece of film. The bands cannot have arisen from digital gel documentation (gel-doc) because the blots were developed using film… to develop using two separate methods (film plus gel-doc) and combine the results is at odds with scientific rationale.

<u>Stage 3.</u> Further anomalies and notable features

Focusing on the right edge of the left-hand blot image in *20H7 & FLNA n-Ter-test9 JR-11.jpg*, application of a gradient map in Adobe Photoshop followed by enlargement of this image reveals a strange box-like feature (corners highlighted in green here on the left ←). The background noise and pixelation inside this box appears to be different to that outside the box. Such sharp changes in background are highly suggestive that part of the image has been pasted in from elsewhere, or that a part of the image has been selected and independently adjusted. This suggests inappropriate manipulation.





Furthermore, application of a JPG Error Level Analysis (ELA) from the website https://fotoforensics.com/ to the white box image (B) *20H7 & FLNA n-Ter-test9 JR-11.jpg* is shown here (← original on top, ELA below). It is generally understood that all parts of a JPG image should be at roughly the same compression and error level. If a section of an image has a significantly different error level, this can indicate it may have originated elsewhere.

ELA reveals that one of the bands in the left-hand blot contains significantly different JPG compression and error information, compared to the rest of the image (this is indicated by the black band annotated with a red arrow). This is highly suggestive that this band has been added from a source image with a different JPG compression level, or this part of the image has been independently manipulated relative to the remainder of the image.

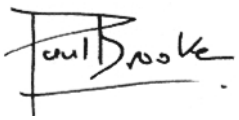
Furthermore, despite the appearance of molecular weight (MW) markers in the final figure, none of the original images contain any information on MW. There are no MW ladders or markings on the film. As such, the origin of the MW marker assignments in the final figure is unclear. Finally, it is notable that both the raw image (A) and white-box image (B) appear to contain smudges, stains, botches, streaks and other undesirable features within the blot images. These are generally indicative of poor-quality western blots, poor experimental practices, or lack of specificity of the antibody being used.

PowerPoint Intermediate File

The .PPT file *"Origene-20H20-20H7+FLNA NT-JR9 test.pptx"* contains the complete Figure 2 in annotated form, with each of the original images noted above pasted into place and matching the final image. The modification date for this file (2019-10-02) is in between the dates of the JPG images (2019-10-01) and the submission of the grant application (2019-11-18).

Summary & Conclusion.

Based on the above evidence, I conclude that the source of the final image (C) in the 3rd row of Figure 2 of the grant proposal is the white box image (B) "*20H7 & FLNA n-Ter-test9 JR-11.jpg*". Although this image and its corresponding raw image (A) "*20H7 & FLNA n-Ter-test9 JR.jpg*" share several common features, application of acceptable image manipulation processes to the raw image was unable to reproduce the bands in the white box image. As such, the band pattern in the final published image finds no provenance in the source images. In the absence of original blot images showing the bands of interest as they appear in the final figure, it is my professional opinion that the final figure and its parent white box image and band pattern have been fabricated. As per the federal definition of scientific misconduct, the presented data do not appear to accurately represent the research record.



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